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Isolation of *Pasteurella* spp. from Sick and Healthy Feedlot Calves Using Four Different Sampling Techniques[1]

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Introduction

Bovine respiratory disease is the most common disease complex of feedlot cattle. The peak incidence of the disease occurs within the first few weeks of arrival at the feedlot. Bovine respiratory disease is attributed to a complex interaction between bacteria, viruses, environment, stress, and managerial practices. *Pasteurella hemolytica*, and to a lesser extent *Pasteurella multocida*, are considered to be the most common bacterial isolates from cases of bovine respiratory disease.

The purpose of this study was threefold: 1) compare the ability of four different sampling techniques to isolate *Pasteurella* spp. from the respiratory tract of calves, 2) compare the prevalence of *Pasteurella* spp. in the respiratory tract of sick calves and clinically normal cohorts, and 3) evaluate the feasibility and practicality of performing nonsurgical tracheal washes in a feedlot setting.

Procedure

In the fall a group of 64 spring born calves ranging in age from four to six mo were weaned and transported to the MARC feedlot. All of the calves were born and raised at MARC. The calves were vaccinated three wk prior to weaning with a modified live infectious bovine rhinotracheitis virus and bovine viral diarrhea virus (IBR-BVD), polyvalent *Clostridium* spp., and polyvalent *Leptospira* spp. vaccines. Calves were boostered with IBR-BVD and given ivermectin 30 days after weaning and transport to the feedlot. In the feedlot the calves were fed chopped brome hay for the first three days, 40% ground alfalfa hay was added to the diet for the following five days, and silage was introduced after eight days.

The calves were monitored for 28 days in the feedlot. The cattle were observed daily for signs of disease and were removed from the pen when signs of disease became apparent. Calves which exhibited rapid breathing, a runny nose, coughing, inappetence, depression, or isolation were removed to the hospital facility. If a removed calf had a rectal temperature of 103°F or above and the illness could not be referred to any other body system, the calf was treated for respiratory disease. The treatment protocol was intravenous tylosin (8 mg/lb) and oxytetracycline (7 mg/lb) daily for four days, oral sulfadimethoxine boluses (62.5 mg/lb) on the first day and intramuscular vitamin B complex (1 ml/100 lb) on the first day. For each sick calf removed from the pen, sampled, and treated a clinically normal cohort of the same approximate age, gender, and disease history was removed from the pen and sampled but not treated.

Sick and cohort calves were sampled on the day they were removed from the pen before the initiation of treatment and on the last day of the initial respiratory disease treatment. The calves were restrained in the treatment chute and a six inch sterile cotton swab was inserted into the nostril. The swab was then placed into a sterile tube with one milliliter of sterile phosphate buffered saline (PBS). A 13.25 inch guarded nasal swab was then inserted into the nostril. A mouth speculum was used to open the mouth and a sterile 26.25 inch guarded tracheal swab was inserted through the mouth and into the trachea. Both the 13.25 and 26.25 inch guarded swabs were closed systems that contained transport media. Finally, a 36 inch piece of eight millimeter diameter rigid plastic tubing was inserted through the mouth and into the trachea. A sterile length of flexible tubing was threaded down the plastic tube into the trachea. Using a 60 milliliter syringe, approximately 120 milliliters of sterile PBS was injected down the flexible tubing into the distal trachea and quickly aspirated back to obtain a tracheal wash. The total sampling time for each calf was approximately two minutes.

The samples were transported four miles to the Great Plains Veterinary Educational Center Clinical Microbiology Laboratory and plated on blood agar with 5% sheep blood and McConkey II agar. The plates were incubated at 98.6°F in 5% CO_2 and examined at 24 and 48 hr for growth. *Pasteurella* spp. were identified using standardized isolation procedures.

Mantel-Haenszel Chi-squares were calculated using a public-domain microcomputer epidemiologic statistic program (USD Inc, Stone Mountain, Georgia).

Results

Of the 64 calves included in the study, 19 developed clinical bovine respiratory disease within the first 28 days of their arrival at the feedlot. Each of the cohorts remained healthy during the sampling period with the exception of one calf which developed respiratory disease and was then included in the sick group. The morbidity rate was 31% and the mortality rate was 0% for this group of calves during the study period. The results of the *Pasteurella* spp. isolation for each sampling technique are summarized in Table 1.

When the four different sampling techniques were compared on each day for cohort and sick calves there was not a significant difference in the number of *Pasteurella* spp. isolates obtained by any of the techniques.

This information is significant because there is a great difference in the degree of difficulty of sample collection between the four techniques. The performance of all four techniques required minimal time, thus all of the techniques can be performed in the feedlot. The easiest and least expensive of the four is carefully placing a six inch cotton swab into the nasal passage, placing it in PBS, and transporting it to the laboratory. Other techniques involved more elaborate and expensive collection systems.

All of the techniques required handling and restraint of the head of the calf, thus all likely caused some degree of psychological distress. Collection of the two nasal samples required a lesser degree of restraint and manipulation than collection of the two tracheal samples, with the tracheal wash requiring the longest and most vigorous restraint.

Care must be taken with all four techniques to properly restrain the calf. If restraint is inadequate the wooden shaft

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of the six inch nasal swab could break, resulting in loss of the cotton tip in the nasal passage. Epistaxis (nosebleed) could be induced if the guarded deep nasal swab was not introduced with care. The mucosal covering of the lyssa of the tongue could be torn if the tongue was restrained too vigorously during collection of tracheal samples. No other adverse effects that could be attributed to sample collection were noted in any calves.

The main consideration for the selection of a sampling technique is its ability to isolate relevant pathogenic microorganisms. Other important considerations include practicality in a field setting, lack of complications, skill required, ease of sample collection, degree of calf distress induced, and cost of sample collection. These data suggest that when all of these factors are considered, a six inch nasal swab is the most effective sample collection system.

It is not known if isolates were *Pasteurella multocida* or *Pasteurella hemolytica*, as only the genus of isolates was characterized. Additionally, if isolates were *Pasteurella hemolytica*, it is not known if isolates were serotype 1 or 2. This information would allow us to better assess the pathogenic relevance of the *Pasteurella* isolates since pneumonic pasteurellosis in cattle is typically associated with *Pasteurella hemolytica* A1.

Samples were collected after calves were identified as sick. Differences in the microflora between the upper and lower respiratory tract may have been overlooked because the sample was taken after the disease process was well advanced. However, these data suggest that during an outbreak of bovine respiratory disease there is no difference between the sampling techniques examined.

On the first day of treatment there was not a significant difference in the number of calves from which *Pasteurella* spp. was isolated between the sick and cohort groups. Also, there was not a significant difference in the number of calves from which *Pasteurella* spp. was isolated in the cohort calves on day one and day four. For the sick calves there was a significant drop in the number of calves from which *Pasteurella* spp. was isolated from day one to day four of treatment. This is likely a result of antimicrobial therapy.

Differentiation of sick and cohort calves was based on subjective criteria. Since nontreated controls were not included in the sick group, it is possible that healthy calves were erroneously included in the sick group. Misclassification of calves could have affected the outcome of this trial. However, as only one of the cohort calves became sick and none died, misclassification seems less likely.

The treatment protocol used was effective in causing a shift in the microflora of the nasopharynx and trachea. All the calves diagnosed as having bovine respiratory disease and undergoing treatment did recover and remained clinically healthy during the remainder of the study period.

These findings are consistent with and extend previous research. The lower respiratory tract of a normal, unstressed calf is usually sterile. Normal, unstressed calves carry low numbers of *Pasteurella* spp. as part of their nasal flora. Due to changes in the respiratory tract induced by husbandry practices such as weaning and transport, or infection with viruses or mycoplasmas, floral shifts occur that result in an increase in the numbers of pathogenic *Pasteurella* in the respiratory tract. This increase in the challenge dose presented to the defenses of the lower respiratory tract, along with compromise of the defense mechanisms of the lower respiratory tract, results in pneumonic pasteurellosis. Antibiotic therapy does not "cure" the animal. Rather it suppresses bacterial proliferation to such a degree that the pneumonic defenses of the calf can clear the infection.

In summary, there was no significant difference in the ability to isolate *Pasteurella* spp. from sick or cohort calves using either a short nasal swab, a long guarded nasal swab, a guarded tracheal swab, and tracheal lavage. There was no significant difference in the isolation of *Pasteurella* spp. between the sick and cohort calves on the first day of treatment. There was no significant difference in the samples obtained from the cohort calves on day 1 and day 4. There was a significant difference in the ability to isolate *Pasteurella* spp. in the sick calves on day 1 compared to day 4 that is likely a result of antimicrobial therapy.

Table 1—Isolation rate for four sampling techniques used on sick and cohort calves on day found sick and after therapy

	Sampling Technique			
Group	Nasal swab	Guarded nasal swab	Tracheal swab	Tracheal wash
Sick				
Day found sick	12/19 (63)[a]	13/19 (68)	10/19 (53)	8/19 (42)
After therapy	2/19 (11)[b]	1/19 (5)[b]	2/19 (11)[b]	1/19 (5)[b]
Cohort				
Day found sick[c]	8/18 (44)	10/18 (56)	9/18 (50)	8/18 (44)
After therapy	9/18 (50)	7/18 (39)	6/18 (33)	5/13 (28)

[a] Data are expressed as number of *Pasteurella* spp. isolates/number of samples (%).

[b] Values differ from other values in column ($P<.05$).

[c] Cohort calves were removed from their home pen and sampled on the same day as sick calves. They received no treatments. They were held in hospital pens and sampled again the last day of the initial respiratory disease treatment of the sick calves.

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